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TEMPERATURE AND HUMIDITY STUDIES OF SOME FUSARIA ROTS OF THE IRISH POTATO [1]

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INTRODUCTION

The ability of *Fusarium oxysporum* Schlect. to cause a rot of the potato tuber has been clearly demonstrated by a number of workers. The influence of temperature on this disease has been reported in a number of papers, but the experimental evidence as a whole is rather meager, usually only extreme temperatures being used. The effect of moisture on the progress of the disease, except under conditions of extreme dryness or saturation, has received practically no attention. It was with the purpose of determining the relation of temperature and humidity to the progress of potato tuber-rots caused by Fusaria that the following work was undertaken.

HISTORICAL

The association of Fusaria with storage-rots of the Irish potato (*Solanum tuberosum* L.) has been a matter of common observation by most workers in plant pathology from 1842 to date. Several species of the form genus Fusarium Link have been described as causes of potato tuber-rots, by Von Martius (*12*), Reinke and Berthold (*18*), Schacht (*19*), Pethybridge and Bowers (*14*),[2] Longman (*10*), and Sherbakoff (*20*). The fact that Fusarium species could produce a rot of the tuber was demonstrated by Pizzigoni (*15*) and Wehmer (*24*, *25*), who described the species they worked with as *Fusarium solani* (Mart). Frank (*6*), De Bary (*2*), and others considered that the Fusaria were unable to produce a rot of the tuber. In most of the earlier papers, *F. solani*, or some species thought to be a synonym of it, was given as the causal organism.

Owing to the absence of clearly defined species in all the literature previous to Appel and Wollenweber's (*1*) monograph on the form genus Fusarium in 1912, no attempt will be made to review in detail the earlier reports of potato tuber-rots caused by Fusaria.

Fusarium oxysporum was considered by Wollenweber (*27*) to be a strictly vascular parasite producing a wilt of the potato vine but not

[1] Published with the approval of the Director of the Nebraska Agricultural Experiment Station. The paper is based upon experimental work undertaken at the Michigan Agricultural College in 1914–15, and at the University of Wisconsin in 1916–17.

[2] Reference is made by number (italic) to "Literature cited," p. 77-79.

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causing a rot of the tuber. Carpenter (*4*) in 1915 was the first to report successful infections by inoculations with pure cultures of *F. oxysporum*. He made these by dipping wounded tubers in a water suspension of spores, wrapping in oiled paper and keeping them at controlled temperatures ranging between 17° and 30° C. No detailed experiments were reported except in this saturated atmosphere. He noted, however, that either a dryrot or a wetrot was produced, according to the temperature and humidity used. He concluded that a constant storage temperature below 50° F. (10° C.) would prevent the action of *F. radicicola* Wollenw., *F. eumartii* Carp., and *F. oxysporum*. Previous to this work of Carpenter's, Smith and Swingle (*22*), in 1910, described a bundle blackening and a dry endrot of the tuber as two stages of the same disease. They attributed this to a Fusarium for which they accepted the name *F. oxysporum* as first applied to it by Schlechtendahl (*21, p. 139*). They noted that the disease continued in stored potatoes and that when potatoes were stored in warm rooms, either moist or dry, they became badly diseased, whereas those stored in cool places kept much better. They did not differentiate this species of Fusarium from others occurring on the potato, and no inoculation experiments were recorded. Manns in 1911 (*11*), working with the same disease, stated that the "dormant internal infection" under improper storage conditions becomes so active as to cause a high percentage of dryrot. He noted that the disease was favored by high temperature and considerable moisture. At 36° to 40° F. (2° to 3° C.) the disease made no progress, at 45° to 55° F. (7° to 12° C.) it developed gradually and caused considerable rot, especially when accompanied by high humidity. He made no mention of pure culture inoculations on tubers or morphological studies.

Jamieson and Wollenweber in 1912 (*8*) described a dryrot of the potato tuber caused by a species of Fusarium which they named *Fusarium trichothecioides* Wollenw. They made inoculation experiments and found the most rapid penetration of the tuber to take place at 10° to 12° C. in an atmosphere of low humidity. Rotting took place at the high humidities but not as rapidly. Wilcox, Link, and Poole (*26*) published on a dryrot of the potato tuber caused by a Fusarium which they called F. *tuberivorum* W. and L. but which was undoubtedly the *F. trichothecioides* previously described by Jamieson and Wollenweber (*8*). They found that a temperature of 8° to 10° C. was only slightly inhibitive to the growth of the fungus and that when potatoes infected with the organism were stored at this temperature, the most rapid decay took place when the humidity was high. Pratt (*17*), working with the same disease, found that temperatures ranging from 12° to 25° C. were favorable for the progress of the disease and that dryrot did not develop at temperatures below 2° C. He concluded from storage experiments that in a dry, well-ventilated storage house losses would be very slight at temperatures from 2° to 4° C.

Link (*9*), making comparative studies of *Fusarium oxysporum* and *F. trichothecioides* found that both were capable of producing a rot of the potato tuber and that *F. trichothecioides* produced a typical dry-rot. *F. oxysporum* produced a softrot of the whole tuber except under cold, dry conditions, when a dryrot was produced. He ran his experiments at controlled temperatures ranging from 1° to 30° C. in an almost saturated atmosphere.

Pratt (*16*) found that *Fusarium radicicola* behaved much the same as *F. oxysporum*, and he concluded from storage experiments that the tuber-rot caused by this organism does not make any progress in storage at a temperature of 48° F. (8.8° C.) or below.

In general, then, it can be said that a high temperature favors the production of tuber-rots by all three of these Fusaria, although *Fusarium trichothecioides* appears to be able to produce a rot at lower temperatures than the other two. High humidities also appear to favor the production of tuber-rot. With the exception of the paper of Jamieson and Wollenweber (*8*) all the evidence points toward an increase in rotting with an increase in humidity.

TEMPERATURE RELATIONS IN PURE CULTURES

A review of the literature shows a general conformity of results regarding the relation of temperature to the growth of *Fusarium oxysporum*. Link (*9*) by making dry-weight determinations of growth in liquid media found 30° C. to be the optimum for growth. Edson and Shapovalov (*5*), working with Petri-dish cultures, obtained the same optimum. They reported a maximum temperature of 37°C., where the spores changed to chlamydospores; they did not observe growth at 5° C. Humphrey (*7*) gives 4° C. as the minimum temperature for certain strains of *F. oxysporum*.

The writer, working with three strains of *Fusarium oxysporum* and using the same methods for measuring growth, obtained somewhat similar results to those reported by Edson and Shapovalov (*5*). The minimum temperature for growth was 9.5° C., no growth taking place at the next lower temperature of 7° C. The maximum temperature was 37.5° C., where there was a very slight growth.

Fusarium trichothecioides is apparently unable to grow at 30° C., which is the optimum temperature for *F. oxysporum*. Link (*9*) found the greatest growth of *F. trichothecioides* in liquid potato extract media at the end of 20 days to take place at 12° C., with no growth present at 30° C., although the organism was capable of living in the potato tuber at that temperature. Edson and Shapovalov (*5*) obtained a much higher optimum for *F. trichothecioides;* they found the greatest growth took place at 25° C., with a sharp drop to the maximum temperature at 30° C., where germination of spores took place but no growth of mycelium.

The writer, working with two strains of *Fusarium trichothecioides* in Petri dishes, found 25° C. to be the optimum temperature, and with one strain he was able to obtain slight growth, 7 mm. in diameter, at the end of one week at 30° C. At 5° C. germination took place and there was slight growth.

The optimum temperature for *Fusarium radicicola* was 30° C., the same as for *F. oxysporum*. The minimum was at 5° C., where a very slight growth was produced in 10 days. At 35° C. the growth was greater than with *F. oxysporum*, although the rate of growth was slower. Edson and Shapovalov (5) report a similar optimum temperature, with germination but no growth at 5° C. They found that at 39° C. a transformation from normal spores to chlamydospores took place.

In general it can be said that at 25° C. the growth for all three species is nearly equal, *Fusarium oxysporum* and *F. radicicola* increasing in growth up to 30° C. and *F. trichothecioides* decreasing. The minimum temperature for *F. oxysporum* is higher than for the other two, and in general *F. trichothecioides* appears to be more tolerant of the lower temperatures than the others.

Preliminary experiments, using liquid media and determining the growth by dry weights, have been conducted with a number of strains of these three species. While on certain media the results have in general corroborated the foregoing cardinal points for growth, they indicated that these cardinal points may vary with the medium used. For instance, with a nutrient solution made up of ammonium nitrate (NH_4NO_3), potassium phosphate (KH_2PO_4), magnesium sulphate ($MgSO_4$), ferric chlorid ($FeCl_3$), and sucrose, the results compared well with those obtained on agar in Petri dishes, With a nutrient solution made up similarly to the potato extract medium used by Link (9), the total growth at the higher temperatures was considerably less than the growth obtained in the first nutrient solution, while at the lower temperatures the growth was much greater. The optimum temperature for growth of *Fusarium trichothecioides* in the first nutrient solution was 25° C., with no growth taking place at 5° C. With Link's potato-extract medium the optimum lay between 15° and 20° C., and there was weighable growth at 5° C. These results would possibly account for the considerable discrepancy between the results obtained by Link (9) with liquid media and those obtained by the writer and by Edson and Shapovalov (5) with agar cultures.

EXPERIMENTAL INFECTION OF TUBERS

The cultures used in the following experiments, with their origin, are listed below. In practically all cases the various strains of the same species behaved alike. Several other strains of *Fusarium oxysporum*, isolated by the writer, were also used in the experiments in addition to the ones listed below.

No. 1.—*Fusarium oxysporum*, isolated by the author from browned vascular bundles of potatoes and identified by H. W. Wollenweber and numbered at Washington as 3377.

No. 8. *Fusarium oxysporum*, obtained from C. W. Carpenter of the United States Department of Agriculture, No. 3395.

No. 32.—*Fusarium oxysporum*, obtained from G. K. K. Link, of the University of Nebraska, as No. 3345a.

No. 28.—*Fusarium trichothecioides* obtained from G. K. K. Link.

No. 31.—*Fusarium trichothecioides*, obtained from A. C. Pratt of the United States Department of Agriculture.

No. 29.—*Fusarium radicicola*, obtained from A. C. Pratt and numbered 716.

METHODS

In all inoculation experiments with tubers, potatoes which were of one variety, of the same age, and had been kept under the same storage conditions were carefully selected for uniformity of size, type, and freedom from wounds. The stem ends always were cut and examined for natural infection, and all tubers showing vascular discoloration were discarded. The tubers were always treated with formaldehyde or mercuric chlorid and washed in sterile distilled water.

The inoculations were made by wounding the epidermis, usually by stabbing to a depth of 3 mm. with a sterile scalpel. The inoculum was introduced in various ways as outlined in the experiments.

EXPERIMENT 1, DECEMBER, 1915.—Potato tubers of the Up-to-Date variety were inoculated by wounding the tubers and then dipping them in a water suspension of spores, wrapping in sterile waxed paper, and placing in moist chambers at 25° C. Controls were treated in the same way, being dipped in sterile water. Results were taken 18 days later.

Set No. 1. Four tubers inoculated with *Fusarium oxysporum* No. 1. All tubers completely rotted. The two control tubers remained sound.

Set No. 2. Four tubers inoculated with *Fusarium oxysporum* isolated from infected tubers in storage. All tubers showed a complete wetrot; the tissue was soft and of a light brown color; a large cavity was present in each tuber containing masses of white mycelium. At the point of inoculation there was a granular mass of hyphae and starch grains separated from the rest of the tissue. Control tubers remained healthy.

Set No. 3. Four tubers inoculated with *Fusarium oxysporum*, isolated from wilted potato vines. All tubers showed a dark brown dryrot progressing only a short distance from the point of inoculation. Controls remained healthy.

Reisolations were made from all the rotted tubers, and *Fusarium oxysporum* was recovered in every case. No bacteria or secondary invaders were found in any of the tubers. These results show that *F. oxysporum* is capable of producing a rot of the tuber in a saturated atmosphere at

25° C. The characteristic rot under these conditions is a soft wetrot with no sharp line of demarkation between the healthy and diseased tissue. The organism appears to be unable to attack whole starch grains, which accumulate in a granular mass with the mycelium, as in set 2. The tuber-rot under these abnormal conditions is not typical of the rots usually found in storage.

EXPERIMENT 2, FEBRUARY 23, 1916.—Further tests were conducted at the same temperature but with a lower relative humidity to test the ability of the organism to cause a rot under conditions not so adverse for the host as in the previous experiment. Tubers of the Up-to-Date variety were inoculated by wounding and then placing a little of the fungus mycelium and spores in the wound. The tubers were then placed in a sterile moist chamber but were not wrapped in paper. Controls were treated and wounded in the same way. The experiment was run at 25° C. Twelve different strains of *Fusarium oxysporum* were used for the inoculations, two tubers being used for each strain. Results were taken after five weeks.

In only one case had the rot extended three-fourths of the length of the tuber. In all the other tubers there was only a slight rotting extending for a short distance from the point of inoculation. The controls remained sound in every case. The tubers were in a saturated atmosphere at the beginning of the experiment, gradually becoming drier until at the end the tubers were considerably dried out. Compared to the preceding test the amount of rotting was very slight, and its inhibition may be directly attributed to the dryness of the air. The slight amount of rot around the point of inoculation would indicate that the fungus progressed a short distance into the tuber at the beginning of the experiment when the humidity was high but was unable to advance further under the drier conditions. This would indicate that the rotting of tubers already started could be checked by submitting the tubers to lower humidities.

EXPERIMENT 3, APRIL 26, 1916.—A further test on the relation of humidity of the atmosphere to the rot of the tuber was started. The inoculations were made as in the previous experiment, and the same variety of potatoes was used.

Set No. 1. The inoculated tubers were placed in sterile chambers, and moist filter paper was placed in the chambers at the start of the experiment to produce a favorable humidity for the initial penetration of the tuber.

Set No. 2. The tubers were placed in moist chambers in which the atmosphere was kept saturated throughout the experiment.

Both sets were kept at a temperature of 25° C. The results were taken after seven weeks. (Table I.)

TABLE I.—*Comparative amount of rot produced by Fusarium spp. under different conditions of relative humidity*

Strain.	Set No. 1.	Set No. 2.
F. oxysporum No. 1	One-third rotted	Entirely rotted.
F. oxysporum No. 8	2-mm. rot	Do.
F. hyperoxysporum	One-third rotted	Half rotted.
Control	Healthy	Healthy.

In all cases where rotting was present the starch grains were not corroded. Culture No. 8 seemed to have a much slower initial growth than the others, thus showing a greater difference between the two sets. In general, it can be clearly seen that the rotting was much greater in set No. 2, where the atmosphere was saturated throughout the experiment. Although the organisms were capable of starting a rot under the moist conditions at the start of the experiment in set. No. 1, they were later considerably checked under the drier conditions.

EXPERIMENT 4, MARCH 15, 1917.—Further infection experiments were started under conditions in which the relative humidity of the atmosphere was controlled by the use of various concentrations of sulphuric acid. Previous experiments conducted at the Michigan Agricultural Experiment Station in 1915 and described under experiment 5, in which the relative humidities were carefully controlled, produced very good results with *Fusarium oxysporum.*

The apparatus used in experiment 5 was not available in 1917, so the relative humidities used in experiments 4 and 4A were determined from the tables given by Stevens (*23*). One-quart Mason jars were used, in which were hung small wire baskets containing the tubers, the acid being placed in the bottom of the jar. Tubers of the Rural New Yorker variety were inoculated as in the preceding experiments. They were then placed in the baskets in the sterilized jars and were sealed with paraffin and placed at the desired temperatures. The experiment was run in duplicate. Three strains of *Fusarium oxysporum*, two of *F. trichothecioides*, and one of *F. radicicola* were used for the inoculations. The temperatures used were 5°, 9°, 16°, and 25° C. While these temperatures varied somewhat during the experiment, the extremes did not in any case overlap. The relative humidities obtained by using sulphuric acid remained fairly constant throughout the experiment. One hundred cc. of each of the acid solutions were used for each jar. At the close of the experiment the specific gravity of the solutions was taken, and the calculated humidity at this time was compared with that at the start, with the result that the one having 1.5 per cent relative humidity had changed to 3.6 per cent, the 33 per cent to 49 per cent, and the 66.5 per cent to 74 per cent. These variations were not considered great enough to cause conflicting results.

The results were taken after seven weeks, and the penetration of the tubers was measured in millimeters, as shown in Table II. The number of individuals was so small that slight discrepancies in the tabulated results are found. *Fusarium trichothecioides* produced a slight rot at lower temperatures than *F. oxysporum* but did not produce as extensive a rot at the higher temperatures. At 5° C. the only rotting found was with one strain of *F. trichothecioides*, at 100 per cent humidity. No rotting was found at the temperature of 9° at the lower humidities, but there was slight rotting at this temperature at the higher humidities, especially with *F. trichothecioides*. It is noticeable that at 9° with the relative humidities of 66 and 100 per cent, the amount of rotting is greater than at the increased temperature of 16°, with the relative humidities of 1 and 33 per cent. The same comparative results are found between the amount of rotting taking place under the several humidities at a temperature of 16° and of 25°. The results do not conform with the report of Jamieson and Wollenweber (*8*), that penetration of the tuber by *F. trichothecioides* is favored by low humidities. The work by Link (*9*) and Wilcox, Link, and Poole (*26*), however, would indicate that more rapid rotting takes place in an atmosphere of high humidity, thus agreeing with the results shown in this experiment. The results of *F. oxysporum* accord well with those obtained in experiment 3.

TABLE II.—*Extent of penetration of tubers in experiment 4*

Temperature.	Approximate relative humidity.	*Fusarium oxysporum.*			*Fusarium trichothecioides.*		*Fusarium radicicola,* Strain 29.
		Strain 1.	Strain 8.	Strain 32.	Strain 28.	Strain 31.	
°C.	*Per cent.*						
5.....	1	0	0	0	0	0	0
	33	0	0	0	0	0	0
	66	0	0	0	0	0	0
	100	0	0	0	0	3 mm.	0
9.....	1	0	0	0	0	0	0
	33	0	0	0	0	0	0
	66	0	1 mm.	0	0	1 mm.	0
	100	0	1 mm.	0	1 mm.	3 mm.	1 mm.
16.....	1	0	0	0	0	0	0
	33	1 mm.	0	0	0	0	0
	66	2 mm.	3 mm.	0	1 mm.	1 mm.	0
	100	10 mm.	10 mm.	0	1 mm.	1 mm.	2 mm.
25.....	1	5 mm.	10 mm.	1 mm.	0	0	1 mm.
	33	10 mm.	15 mm.	3 mm.	0	2 mm.	6 mm.
	66	20 mm.	½ rot.	½ rot.	1 mm.	25 mm.	6 mm.
	100	½ rot.	Complete rot.	¾ rot.	1 mm.	½ rot.	Complete rot.

EXPERIMENT 4 A, MAY 16, 1917.—In order to check up the possible error due to differences in the age of the tubers used in the various tests, the following experiment was started. New tubers of the Bliss Triumph variety were used in comparison with tubers of the same variety that had

been kept in cold storage from the previous year. The experiment was conducted in the same way as experiment 4, and the same cultures of *Fusarium oxysporum*, *F. trichothecioides*, and *F. radicicola* were used for inoculations. Only two temperatures were used, 13.5° and 25° C., as well as two humidities, 33 and 100 per cent at each temperature. The results shown in Table III were taken after six weeks.

As in experiment 4 the rotting was much greater at the high temperatures and the high humidities. At the lower temperature of 13.5° C. there was no distinct difference between the amount of rotting in the old and new tubers, due to the very slight penetration at this temperature. In the old tubers at 25° the infection in every case had been rapid and the rotting had progressed much further than in the new tubers. These results support the statement of Bisby (*3*) that old tubers are more susceptible to rot than new tubers.

TABLE III.—*Extent of penetration of old and new tubers*

Temperature.	Approximate relative humidity.	Tubers.	*Fusarium oxysporum.*			*Fusarium trichothecioides.*		*Fusarium radicicola*, Strain 29.
			Strain 1.	Strain 8.	Strain 32.	Strain 28.	Strain 31.	
°C.	*Per cent.*							
13.5	33	New.	2 mm.	5 mm.	1 mm.	4 mm.	2 mm.	5 mm.
		Old.	2 mm.	2 mm.	1 mm.	7 mm.	10 mm.	5 mm.
	100	New.	5 mm.	5 mm.	1 mm.	2 mm.	2 mm.	5 mm.
		Old.	5 mm.	4 mm.	1 mm.	5 mm.	6 mm.	5 mm.
25	33	New.	20 mm.	2 mm.	3 mm.	4 mm.	5 mm.	¼ rot.
		Old.	Contaminated.	15 mm.	15 mm.	¼ rot.	5 mm.	¼ rot.
	100	New.	Contaminated.	¼ rot.	Contaminated.	2 mm.	2 mm.	¼ rot.
		Old.	5 mm.	½ rot.	15 mm.	10 mm.	½ rot.	Complete rot.

EXPERIMENT 5, 1915.—The results of earlier experiments having indicated that the influence of the relative humidity was nearly as great as that of temperature, it was decided to run a more complete test on the effect of the relative humidities at different temperatures. Since no apparatus was available by which the relative humidity and temperature could be controlled at will, it was necessary to construct one.

The principle employed in experiment 4 of using sulphuric-acid solutions of varying specific gravity in a closed chamber to obtain the different relative humidities was not used in this test. In preliminary experiments conducted in the same way as experiments 4 and 4A, the infection usually resulted in a softrot which gave good comparative results, but the type of rotting was not similar to that usually found in storage. Cultures from these softrots invariably yielded the *Fusarium* sp. used in the inoculation, and no bacteria were present in any case. Apparently

the absence of any aeration was the cause of this abnormal type of rotting, and the following method was devised to allow for aeration.

The principle finally decided upon was that of passing a current of air, kept at a constant pressure, through sulphuric-acid towers and then over calcium chlorid and sodium hydrate. This gave a constant stream of dry, sterile air. The air was then passed over sterile water to bring it to a desired humidity. The amount of water necessary for a given humidity was determined by trials, and the air was then passed into the jars containing the tubers. An outlet was provided at the bottom of the jar. These jars were connected separately with the current of air and not in series. Relative humidities were obtained and used throughout the experiment as follows: 1, 30, 70, and 100 per cent. These relative humidities were used at three different temperatures—9°, 12.5°, and 25° C. The set at 25° was placed in an incubator in the laboratory, the set at 12.5° was placed in a special low temperature incubator, and the set at 9° was placed in a well-insulated ice box. Each of these temperatures was maintained within a variation of 2° throughout the experiment. In this way four gradations of humidity at each of three temperatures were obtained. The method provided the tubers with sufficient aeration and secured sterile conditions throughout the experiment, since the jars containing the tubers were not moved or opened until the end of the period.

The humidity readings were taken by the wet- and dry-bulb method, the thermometers being inserted into the stream of air at the entrance to the jar. The readings were found to vary, and at least 10 trial readings were taken for each jar after the preliminary determinations were made and the apparatus was set up. These readings ranged as follows: 1 to 10 per cent, 20 to 40 per cent, 60 to 80 per cent, and 90 to 100 per cent. These were the greatest extremes found; and since a knowledge of the approximate relative humidity is all that is necessary in an experiment of this kind, these readings were taken to be sufficient, inasmuch as they showed a gradual gradation from approximate dryness to saturation. The ranges given above simply denote the possible error due to the method of taking the readings. The humidity necessarily remained constant, since the temperature, water surface, and air pressure were constant. It was found to be impossible to use the wet- and dry-bulb method to determine the relative humidity at the lower temperatures. The changes of temperature caused by opening the door to make the determinations were found to change the readings. Therefore the sets at 9° and 12.5° C. were installed temporarily at 25°, the preliminary determinations were made, and the readings were taken at that temperature and corrections made by the use of psychometric tables (*13*).

The large battery jars were fitted with wire screen supports, and six tubers were used in each jar—four inoculated and two controls. The

control tubers were separated from the inoculated ones by a thin layer of cotton. The entire apparatus was disinfected with formaldehyde gas before the experiment was set up.

The tubers used were of the Up-to-Date variety. They had been kept over winter in a cool cellar, and a few sprouts which had started were removed. They were inoculated by wounding the epidermis and placing several drops of spore suspension in the wound. They were then placed in the jars which were closed with cork tops and paraffined. The inoculations were made with *Fusarium oxysporum* No. 8. The jars were opened up and the tubers examined after five weeks.

Set 1 (9° C.). At 10 and 30 per cent humidity the tubers were all healthy.

At 70 per cent, the tubers were sound with no penetration, although there was a slight growth of mycelium on the surface of the tuber at the point of inoculation.

At 100 per cent, the condition of tubers was the same as at 70 per cent, except that the external growth of mycelium was greater. All the control tubers of this set remained healthy, and both the controls and inoculated tubers had sprouted.

Set 2 (12.5° C.). At 10 per cent humidity the tubers were healthy. There was no invasion of the tissues.

At 30 per cent, same as above with a slight external growth of mycelium at the point of inoculation.

At 70 per cent, the tubers were about the same as at 30 per cent. (Pl. 10, A.)

At 100 per cent, invasion of the tissue had taken place for about 2 mm. beyond the wound, causing a slight browning of the tissue. On the surface there was a slight brown discoloration for several millimeters surrounding the point of inoculation and a slight growth of aerial mycelium. All the control tubers in set 2 remained healthy, and both control and inoculated tubers were sprouting normally. (Pl. 10, B.)

Set 3 (25° C.). The control tubers remained healthy and sprouted at 10 and 30 per cent relative humidity, while at 70 and 100 per cent there was a slight disorganization of the tissue around the eyes and the sprouts were all dead. No actual rotting was present or any fungus growth.

With infected tubers at 10 per cent humidity all inoculations were successful and uniform. The fungus invaded the tissue for 2 cm. around the wound. Immediately below the surface at the point of inoculation there was in every case a cavity lined with a white mycelial growth. The tissue surrounding the cavity was of a granular appearance. Examined under the microscope it appeared to be made up of a tangled mass of mycelium and starch grains. A softrot extended out from this area, the tissue being light brown in color and completely invaded by mycelium (Pl. 10, C).

At 30 per cent humidity the rotting took place in the same manner as at 10 per cent, except that the cavity was larger and the rot extended through about 50 per cent of the tuber (Pl. 11, B).

At 100 per cent humidity there was a total rot of all tubers, most of the surface being covered with a white mycelial growth (Pl. 11, C).

The results of this experiment show very clearly that relative humidity plays a very important part in determining the amount of rot produced by *Fusarium oxysporum*. The only rot appearing at the low temperature of 12.5° C. was in an atmosphere of 100 per cent humidity. Even at the high temperature of 25° complete rotting did not take place at the lower humidities. A gradual increase in the amount of rot corresponding to the increase in humidity was present in every case. The fungus can live and sporulate at the lower temperatures and lower humidities used in this experiment but apparently is not capable of penetrating the tubers under these conditions. It can be safely concluded that *F. oxysporum* under good storage conditions is not capable of producing a tuber-rot of great importance.

CONCLUSIONS

(1) *Fusarium oxysporum*, *F. trichochecioides*, and *F. radicicola* are all capable of producing a rot of the potato tuber.

(2) In pure culture the amount of growth of all three species is nearly equal at 25° C., *Fusarium oxysporum* and *F. radicicola* increasing in growth up to 30°, where they produce their maximum growth. The growth of *F. trichochecioides* decreases above 25°, until at 30° very little or no growth takes place. It is more tolerant of the lower temperatures than the other two species.

(3) Preliminary tests with different liquid media would indicate that the cardinal points for growth of these Fusaria vary to some extent with the medium used.

(4) Experimental infection of tubers was produced with all three organisms under various conditions of temperature and relative humidity.

(5) Preliminary tests with *Fusarium oxysporum* indicated that the relative humidity plays a very important part in determining the amount of rotting.

(6) In comparative tests with new and old tubers there is a distinct difference in the amount of rotting under the same conditions. The rotting was much more rapid and progressed much further in the old than in the new tubers.

(7) Comparative tests with all three species at controlled relative humidities from 1 to 100 per cent and at controlled temperatures from 5° to 25° C. proved conclusively that—

(*a*) A temperature of 25° C. is favorable for the production of a tuber rot by *Fusarium oxysporum*, *F. radicicola*, and *F. trichothecioides*.

(*b*) *Fusarium oxysporum* grows more rapidly and produces a more extensive rotting of the tuber than the other two at a temperature of 16° C. and above.

(*c*) *Fusarium trichothecioides* is capable of producing a rot at much lower temperatures than the others, in some cases causing rotting at 5° C.

(*d*) The relative humidity plays a very important rôle in determining the progress of tuber rots and has the same influence on all three species. In every experiment it was noticeable that there was a gradual increase in the amount of rot corresponding to an increase in relative humidity. With a high humidity at a given temperature the rotting was always greater than at a temperature 5° to 10° C. higher but with a low humidity. The Fusaria used can all live and sporulate at the low temperature of 9°, and with low relative humidities, but they are not capable of producing a rot under these conditions.

Inasmuch as the three species of Fusaria used in these experiments represent the common types causing storage-rots of potatoes, it is clear that considerable attention should be given to moisture as well as temperatures where incipient rot occurs in stored tubers. It is also entirely probable that a rotting of the tubers initiated at high temperatures and high relative humidities could be completely checked by submitting the tubers to lower temperatures and lower humidities.

LITERATURE CITED

(1) Appel, Otto, and Wollenweber, H. W.
1910. Grundlagen einer monographie der gattung fusarium (link). Arb. K. Biol. Anst. Land. u. Forstw., Bd. 8, Heft 1, 207 p., 10 fig., 3 pl. (1 col.) Verzeichnis der wichtigsten benutzten Schriften, p. 196–198.

(2) Bary, Anton de.
1861. Die gegenwärtig herrschende kartoffelkrankheit, ihre ursache und ihre verhütung. Eine pflanzenphysiologische untersuchung . . . 75 p., 1 pl. Leipzig.

(3) Bisby, G. R.
1919. Studies on fusarium diseases of potatoes and truck crops in Minnesota. Technical. Minn. Agr. Exp. Sta. Bul. 181, 58 p., 30 fig. Bibliography, p. 40–44.

(4) Carpenter, C. W.
1915. Some potato tuber-rots caused by species of fusarium. *In* Jour. Agr. Research, v. 5, no. 5, p. 183–210, pl. A–B (col.), 14–19. Literature cited, p. 208–209.

(5) Edson, H. A., and Shapovalov, Michael.
1920. Temperature relations of certain potato-rot and wilt-producing fungi. *In* Jour. Agr. Research, v. 18, no. 10, p. 511–524, 9 fig.

(6) Frank, Albert Bernhard.
1898. Untersuchungen über die verschiedenen erreger der kartoffelfäule. *In* Ber. Deut. Bot. Gesell., Bd. 16, Heft 8, p. 273–289.

(7) Humphrey, H. B.
1914. Studies on the relation of certain species of fusarium to the tomato blight of the Pacific Northwest. Wash. Agr. Exp. Sta. Bul. 115, 22 p., 5 pl.

(8) Jamieson, C. O., and Wollenweber, H. W.
1912. An external dry rot of potato tubers caused by fusarium trichothecioides, wollenw. *In* Jour. Wash. Acad. Sci., v. 2, no. 6, p. 146–152, 1 fig.

(9) Link, George K. K.
1916. A physiological study of two strains of fusarium in their causal relation to tuber rot and wilt of potato. *In* Bot. Gaz., v. 62, no. 3, p. 169–209, 13 fig. Literature cited, p. 207–208. Reprinted as Nebr. Agr. Exp. Sta. Research Bul. 9. 1916.

(10) Longman, Sibyl.
1909. The dry-rot of potatoes. *In* Jour. Linn. Soc. (London), Bot., v. 39, no. 270, p. 120–129, pl. 10. Literature referred to in the text, p. 129.

(11) Manns, Thomas F.
1911. The fusarium blight (wilt) and dry rot of the potato. Preliminary studies and field experiments. Ohio Agr. Exp. Sta. Bul. 229, p. 229–336, 15 pl. (in text).

(12) Martius, Carl Friedrich Philipp von.
1842. Die kartoffel-epidemie der letzten jahre oder die stockfäule un räude der kartoffeln, geschildert und in ihren ursachlichen verhältnissen erörtert. 70 p., 3 col. pl. München.

(13) Marvin, C. F.
1900. Psychrometric tables for obtaining the vapor pressure, relative humidity, and temperature of the dew-point . . . U. S. Dept. Agr. Weather Bur. Bul. 235, 84 p., 2 fig.

(14) Pethybridge, George H., and Bowers, E. H.
1908. Dry rot of the potato tuber. *In* Econ. Proc. Roy. Dublin Soc., v. 1, pt. 14, p. 547–558, pl. 48.

(15) Pizzigoni, A.
1896. Cancrena secca et umida delle patate. *In* Nuovo Gior. Bot. Ital., n. s. v. 3, fasc. 1, p. 50–53.

(16) Pratt, O. A.
1916. A western fieldrot of the Irish potato tuber caused by fusarium radicicola. *In* Jour. Agr. Research, v. 6, no. 9, p. 297–310, pl. 34–37.

(17) ———
1916. Control of the powdery dryrot of western potatoes caused by fusarium trichothecioides. *In* Jour. Agr. Research, v. 6, no. 21, p. 817–832, pl. 108.

(18) Reinke, Johannes, and Berthold, Gottfried.
1879. Die zersetzung der kartoffel durch pilze. 100 p., 9 pl. Berlin. (Untersuch. Bot. Lab. Univ. Göttingen. Heft 1.)

(19) Schacht, Hermann.
1856. Bericht an das königliche landes-oekonomie-collegium über die kartoffelpflanze und deren krankheiten . . . 29, 11 p., 10 pl. (6, 8, 9 col.). Berlin.

(20) Sherbakoff, C. D.
1915. Fusaria of potatoes. N. Y. Cornell Agr. Exp Sta. Mem. 6, p. 87–270, 51 fig., 7 pl. (col.). Literature cited, p. 269–270.

(21) Schlechtendal, D. F. L. de.
1824. Flora berolinensis, pars secunda. cryptogamia. Berlini.

(22) Smith, Erwin F., and Swingle, Deane B.
1904. THE DRY ROT OF POTATOES DUE TO FUSARIUM OXYSPORUM. U. S. Dept. Agr. Bur. Plant Indus. Bul. 55, 64 p., 2 fig., 8 pl. Literature, p. 61–62.

(23) Stevens, Neil E.
1916. A METHOD FOR STUDYING THE HUMIDITY RELATIONS OF FUNGI IN CULTURE. *In* Phytopathology, v. 6, no. 6, p. 428–432. Literature cited, p. 432.

(24) Wehmer, C.
1896. UEBER DIE URSACHE DER SOGNENANNTEN "TROCKENFÄULE" DER KARTOFFELKNOLLEN. *In* Ber. Deut. Bot. Gesell., Bd. 14, Heft 3, p. 101–107, 3 fig.

(25) ———
1897. UNTERSUCHUNGEN ÜBER KARTOFFELKRANKHEITEN. 2. ANSTECKUNGSVERSUCHE MIT FUSARIUM SOLANI (DIE FUSARIUM-FAÜLE.) *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 3, No. 25/26, p. 727–743, pl. 10–11 (1 col.).

(26) Wilcox, E. Mead, Link, George K. K., and Poole, Venus W.
1913. A DRY ROT OF THE IRISH POTATO TUBER. Nebr. Agr. Exp. Sta. Research Bul. 1, 88 p., 15 fig., 28 pl. (1 col.). Bibliography, p. 85–88.

(27) Wollenweber, H. W.
1913. STUDIES ON THE FUSARIUM PROBLEM. *In* Phytopathology, v. 3, no. 1, p. 24–50, 1 fig., pl. 5. Literature cited, p. 46–48.

PLATE 10

Tubers inoculated with *Fusarium oxysporum* and kept for five weeks at the following temperatures and humidities:

A.—12.5° C., 70 per cent relative humidity.

B.—12.5° C., 100 per cent relative humidity.

C.—25° C., 10 per cent relative humidity.

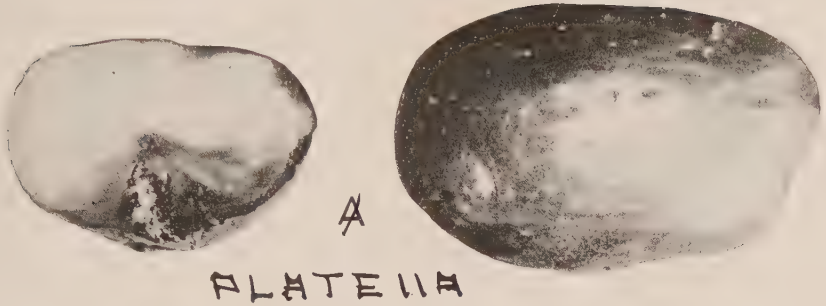
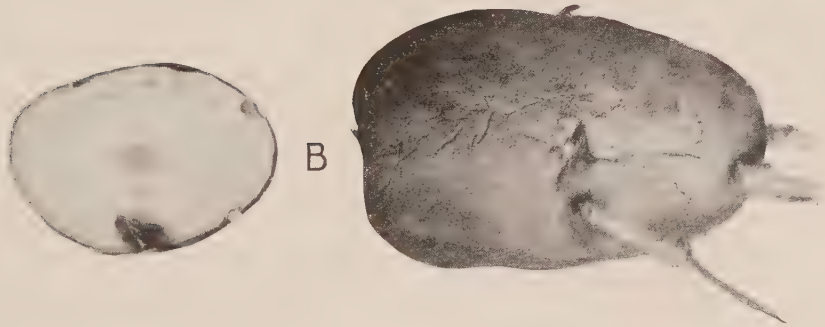


PLATE IIA



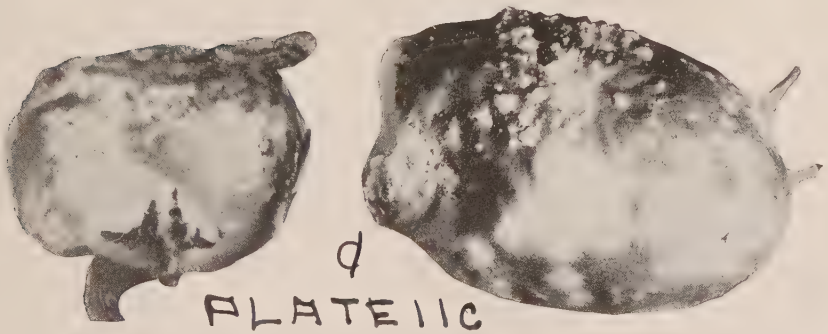


PLATE IIC

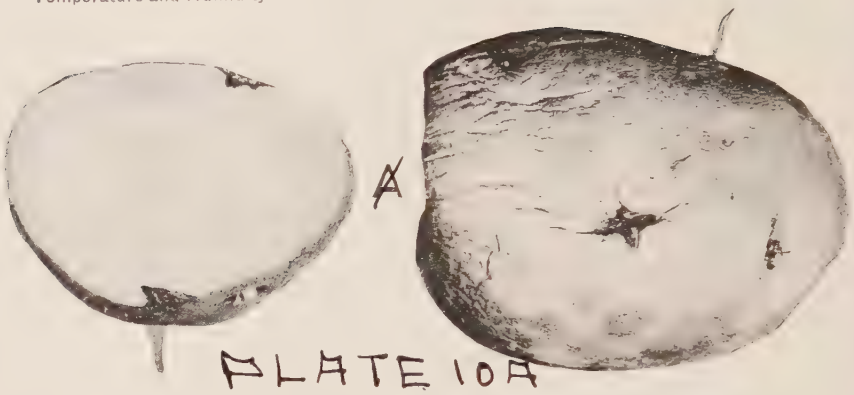

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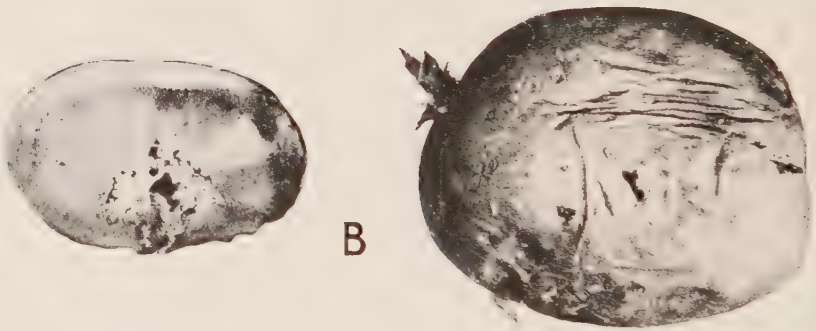
PLATE 10A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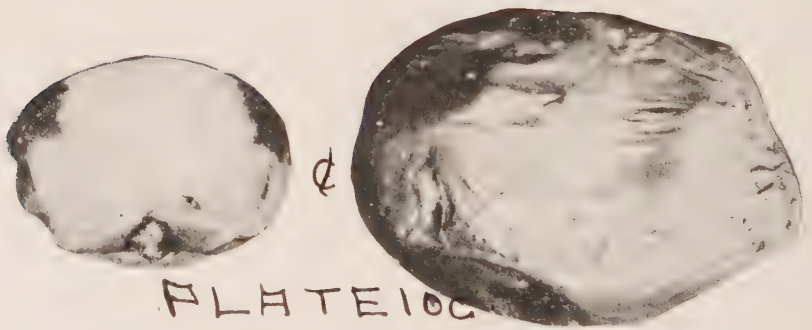
C
PLATE 10C

PLATE 11

Tubers inoculated with *Fusarium oxysporum* and kept for five weeks at the following temperatures and humidities:

A.—25° C., 30 per cent relative humidity.
B.—25° C., 70 per cent relative humidity.
C.—25° C., 100 per cent relative humidity.